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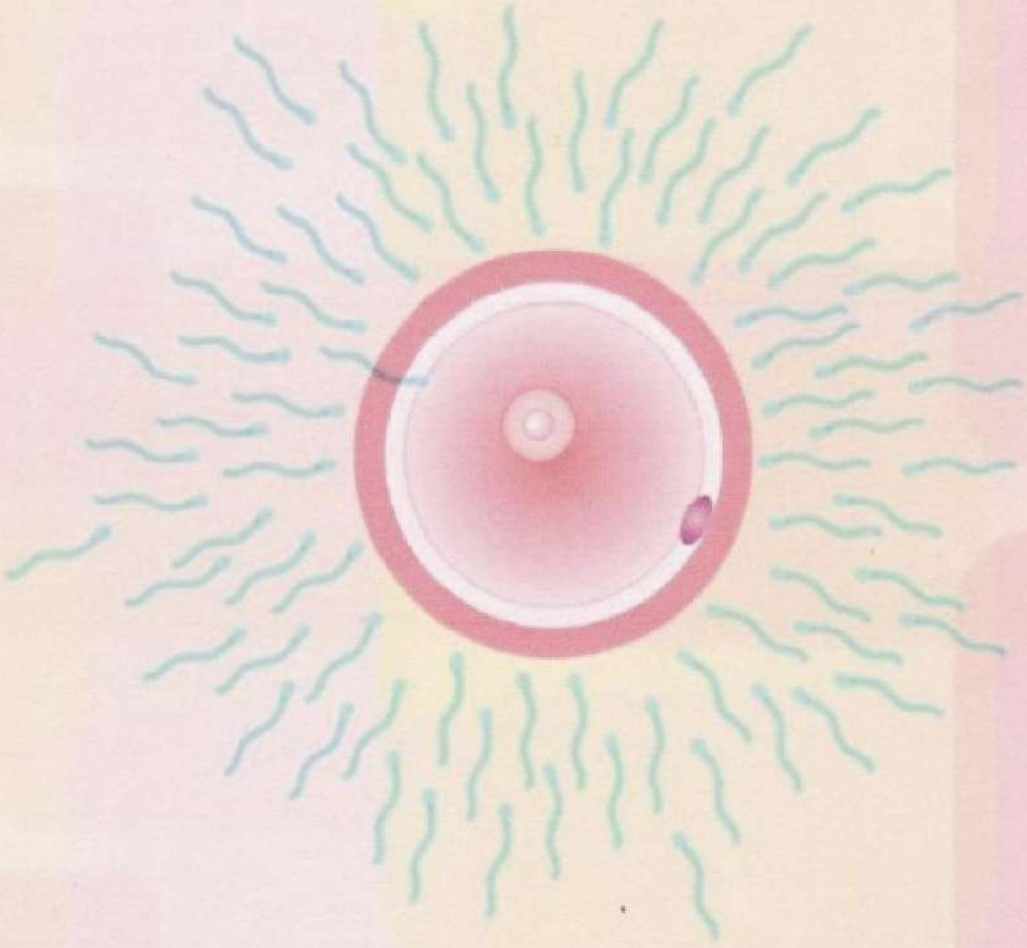

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EFFECT OF "*Sarang Semut*" (*Myrmecodia pendans*) ON THE NUMBER OF SPERMATOGENIC CELLS IN SEMINIFEROUS TUBULES OF MICE (*Mus musculus*) WITH EXCESSIVE PHYSICAL TREATMENT

PENGARUH "Sarang Semut" (*Myrmecodia pendans*) TERHADAP JUMLAH SEL SPERMATOGENIK DALAM TUBULUS SEMINIFERUS MENCIT DENGAN LATIHAN FISIK YANG BERLEBIHAN

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ABSTRACT

This research was to evaluate the number of spermatogenic cells include spermatogonium, spermatocytes, spermatids and spermatozoa after treatment with Sarang Semut + swim stressor as the excessive physical treatment. Treatments were divided into five groups (P0, P1, P2, P3 and P4); where P0 as a control were not given Sarang Semut + without swim stressor, P1 were not given Sarang Semut + swim stressor , P2 were given 15% of Sarang Semut + swim stressor, P3 were given 30% of Sarang Semut + swim stressor and P4 were given 45% of Sarang Semut + swim stressor. The statistical analysis were using ANOVA test and Duncan with Statistical Programe for Social Science (SPSS) program version 16.0 to know the comparison number of spermatogenic cells. The number of spermatogenic cells showed significant differences between treatments ($p<0,05$). The conclusion of this study showed that 15% of Sarang Semut and swim stressor were able to increase the number of sprematogenic cells.

Keywords : Mus musculus, Myrmecodia pendans, spermatogenic cells, excessive physical treatment

Introduction

Free radicals are a chemical group of atoms or molecules that have unpaired electrons in outer layer (Droge, 2002). The existence of unpaired electrons caused them to be very reactive to get its pair, by attacking and binding the molecules of the electron in the vicinity, and when this chemical compound get the new radical, the new radical will be formed and so on, so the chain reaction will be happen. Most of free radicals which are formed in the body caused disturbance to the biomolecules that can impact for the disturbance of structure and function of cells too, which eventually lead to disturbances in work of all organ systems (Winarsi, 2007).

Free radicals may cause the disturbance of human reproductive system. The presence of free radicals may cause 30-80% of the spermatozoa infertile cases (Tremellen, 2008). The sources of free radicals are from metabolism of the body (internal factors), smoke cigarette, chemicals in food or beverage, chemical fertilizers, radiation of Ultra Violet rays, X-ray, and smoke or pollutans from vehicle or plant.

These free radicals will affect to disturbance of spermatogenesis process and sperm membrane, so it will reduce sperm motility and ability to penetrate the ovum (Sutarina & Edward, 2004). Free radicals can also cause DNA disturbance in spermatozoa, especially DNA integrity of the core that can lead to cell death (Tremellen, 2008; Aitken dan Krausz, 2001).

Excessive physical treatment cause an oxidative stress that can reduce the number and motility of spermatozoa (Binekada, 2002; Manna *et al.* 2007). Research about excessive physical treatment (physical

stress) followed with decreasing of sperm quality shows that the Reactive Oxygen Species (ROS) in seminal plasma increased and protection by antioxidants reduced (Tremellen, 2008). The cytoplasmic of spermatogenic cell contains a small amount of scavenging enzymes, but this intracellular antioxidant enzymes were not able to protect the plasma membrane that surrounding the acrosome and tail from free radical attack.

Antioxidant has very important function for the body, because the anti-oxidants are able to reduce the negative impact of the oxidants in the body. There are two kinds of antioxidant they are endogenous and exogenous antioxidant. Endogenous antioxidant enzim such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH-Px), where as exogenous antioxidant such as vitamin E, vitamin C, β-carotene, flavonoids, uric acid, bilirubin and albumin. Utilization of exogenous antioxidant compounds effectively necessary to prevent oxidative stress. Exogenous antioxidant is a preventative measure, where the antioxidant systems worked by intercepting the chain reaction of free radical oxidation or by detaining it (Winarsi, 2007).

Immune system that can be used to fight the free radicals influenced by the availability of nutrients from food that has antioxidant potential. The effort to maintain the high levels of endogenous antioxidants in the body tissues is by consuming foods with high antioxidants. *Sarang Semut* is reported to have flavonoid bioactive compounds which is as an antioxidant (Toda and Shirataki, 1999). The antioxidant activity of flavonoid is determined by the number and position of the aromatic hydroxyl groups that can donate a hydrogen ion (Toda and Shirataki, 1999), and as a detainer (scavenger) of the free radicals that formed during lipid peroxidation (Nijveldt *et al.*, 2001; Amic *et al.* ,2003; Heim *et al.*, 2002).

Subroto and Hendro (2006) showed that this plant contains the chemical compounds of the flavonoid and tannin. Flavonoids are known to detain the free radicals or as natural antioxidants (Amic *et al., 2003;* Lugasi *et al.* 2003).

From the research of Lusiyawati (2008), tannin can affect to the sperm quality, because tannin is chelator that capable to bind the ion particles, such as able to bind the key enzymes in protein synthesis, protein agglomeration and the formation of protein complex compounds with high energy phosphate, so that the phosphate in the body becomes inactive. This results is to decrease the energy of metabolism and nutritional quality which is needed by the semen will be reduce too, so that the quality of sperm motility and viability will be reduced and abnormalities and mortality will be increased.

Research Methods

Material and Equipment

The material used in this research were *Sarang Semut (Myrmecodia pendans),* complete chicken feed CP 593 (PT Charoen Pokhpand Indonesia), drinking water taps, chloroform, distilled water, alcohol 70%, physiological saline, formalin 10% and cotton.

The equipments used in this research were a cage of mice, syringes, surgical instruments (forceps, scalpels, scissors), sonde tools, masks, gloves, pipettes, erlenmeyer, tube reaction, tube rack, petri dish, pail of water, meal box and drink mice, tissue, writing paper and pens to take notes.

The animal used in this study were 20 mice *(Mus musculus)* strain male Balb / C, 10 weeks old, and the weight about 20-25 grams were obtained from the Center for Veterinary Farma (PUSVETMA) and was consisted of 5 treatments to obtain the number of replications of at least 4 mice and overall requires a minimum of 20 male mice.

Methods of research

Mice *(Mus muculus)* were taken randomly and were divided into five treatment groups (P0, P1, P2, P3 and P4) respectively using four replications. The mice were kept in a cage, the feed and drinking water were given *ad libitum.*

The control group (P0) was given only aquadest. P1 group were treated excessive physical treatment. The treatment groups were fed with *Sarang Semut* which dissolved in 100 ml water according to the

dose of *Sarang Semut* 15% (P1), 30% (P2) and 45% (P3) (Sukardiman, 2013). The *Sarang Semut* given orally until day 35. Male mice testes was then collected at the age of 36 days.

After treatment in experimental animals has been done, the *euthanasia* were done. *Euthanasia* conducted on mice aged 36 days by using diethyl ether and then performed surgery to retrieve balls. After that the testes was taken, then put into pots containing 10% formalin conducted by making preparations with *Hematoxylin Eosyn* (HE) staining. The making of the histopathology preparations was performed at the Department of Veterinary Pathology of Veterinary Medicine, Universitas Airlangga.

Microscopic observation of the testes of mice used a microscop with a 100 times magnitude and continued with a 400 times magnitude, after that the number of sperm cells that has increased in the seminiferous tubules of the mice testes were counted.

In this research, the observed variable was dependent variable, that was counting the number of spermatogenic cells include spermatogonium, spermatocyte, spermatid and spermatozoa that had increased the amount, by observing the seminiferous tubules and the number of spermatogenic cells in each testis was then counted. Seminiferous tubules that has increased number of spermatogenic cells were appear more solid and intact. seminiferous tubules that had not treatment were not increase the number of spermatogenic cells and the seminiferous tubules were appear empty and holes.

Data Analysis

Experimental design was used is completely randomized design (RAL) due to the content of the environment and random sampling. In this design there were only one source of variability, that was the random effect of treatment on the side, so the different result of the treatment was only caused by the treatment's effect and random effect. The data analysis was used a statistical test ANOVA and continued with Duncan's test using SPSS *(Statistical Programs for Social Scientifis)* version 16.0. (Kusriningrum, 2008).

Results and Discussion

Spermatogonium

Results of one-way analysis of variance *(One-way analysis of variants)* showed significant differences due to F count> F (0.05) then performed with Duncan's test. The results showed that P4 had the highest score and significant differences from P0, P1, P2, and P3. Treatment P0 had significant differences with P1 and and P4 but not significantly differences with P2 and P3.. P1 had significant differences with P0, P2, P3, and P4. Treatment P2 and P3 had not significant differences but there were significant differences with P0, P1, and P4.

Treatment result from P2, P3 and P4 showed that spermatogonium cells reduced of damage, those could be seen that there were enhancement of spermatogonium cells number compared to the P1 as the positive control. The results of the treatment of *Sarang Semut* best shown in P with treatment of 45% dose of *Sarang Semut* with mean about 85.15.

Spermatocytes

Results of one-way analysis of variance *(One-way analysis of variants)* showed significant differences due to F count > F (0.05) then performed with Duncan's test. The results showed that P4 had the highest score and significant differences from P0, P1, P2, and P3. Treatment P0 had significant differences with P1, P2, P3, and P4. Treatment P1 had significant differences with P0, P2, P3, and P4. Treatment P2 and P3 had no significant differences but there were significant differences with P0, P1, and P4.

Treatment result from P2, P3 and P4 showed that spermatocytes cells reduced of damage, those could be seen that there were enhancement of spermatogonium cells number compared to the P1 as the positive control. The results of the treatment of *Sarang Semut* best shown in P4 with treatment of 45% dose of *Sarang Semut* with mean about 150.35.

Spermatids

Results of one-way analysis of variance *(One-way analysis of variants)* showed significant differences due to F count > F (0.05) then performed with Duncan's test. The results showed that P3 and P4 had the highest score with no

significant differences but significant differences from P0, P1, and P2. Treatment P0 had significant differences with P1, P2, P3, and P4. Treatment P1 had significant differences with P0, P2, P3, and P4 and treatment P2 had significant differences with P0, P1, P3 and P4.

Treatment result from P2, P3 and P4 showed that spermatids cells reduced of damage, those could be seen that there were enhancement of spermatids cells number in P2 nd P3 compared to the P1 as the positive control. The results treatment of *Sarang Semut* best shown in P3 and P4 with treatment of 30% and 45% dose of *Sarang Semut* with mean about 72.95 and 73.45.

Spermatozoa

Results of one-way analysis of variance *(One-way analysis of variants)* showed significant differences due to F count > F (0.05) then performed with Duncan's test. The results showed that P4 had the highest score with significant differences from P0, P1, P2, and P3. Treatment P0 had significant differences too with P1, P2, P3, and P4. Treatment P1 had significant differences too with P0, P2, P3, and P4 and treatment P2 had significant differences too with P0, P1, P3 and P4.

Treatment result from P2, P3 and P4 showed that spermatozoa cells reduced of damage, those could be seen that there were enhancement of spermatozoa cells number in P2, P3, and P4. The results treatment of *Sarang Semut* best shown in P4 with treatment of 45% dose of *Sarang Semut* with mean about 87.10.

Analysis results of spermatogenesis quantitatively, including the number spermatogonia, spermatocytes, spermatids and spermatozoa prove that *Sarang Semut* in multiple doses increased the number of spermatogenic cells. *Swim stressor* as the excessive physical treatment choosed as the free radical that can increase of ROS (Reactive Oxygen Species) because another could damage the spermatogenesis process, it did not affect to the environmental disturbance like smoke cigarette, pollutant from vehicle or plant, chemical fertilization and radiation of Ultra Violets rays that were not safe for the environmental. The treatment with *swim stressor* without being given therapy of *Sarang Semut* showed that the number of spermatogenic cells become decreased. Generally, according to Sagi (1994) testicular activity of spermatogenesis affected by internal and external factors. Internal factors such as body temperature, location of testis and pituitary control. External factors that could influence the spermatogenesis process was psychological excitatory, and environmental changes such as temperature environment, food, certain chemicals, and social contacts. In the excessive physical examination would raise the ACTH secretion and decrease the concentration of LH plasma. After the excessive physical examination, the corticotrophin releasing hormone (CHR) induced the releasing of ACTH and β endorphin. The increasing of β endorphin could inhibit the releasing of gonadotropin (LH secretion) (Safarinejad, *et al.*, 2009). The reducing of LH secretion followed by reducing of testosterone hormone that produced by leydig cells (Colon, 2007).

FSH worked by stimulating the sertoli cells to produce Androgen Binding Protein (ABP). The appearance of FSH began the proliferation process of spermatogenesis and testosterone that diffuse from interstitial cells that required for maturation of late spermatozoa (Guyton, 1991). The inhibition of FSH and LH formation could influence the abnormality of spermatogenesis process. Flavonoid in *Sarang Semut* as an antioxidants would repair the spermatogenesis process to against free radicals, so that the chain reaction would stop and the endocrine system will be protected from damage and the pituitary gland that produce hormones such as FSH and LH become normal.

Conclusion

Based on the research that has been done, it could be concluded that *Sarang Semut* (*Myrmecodia pendans*) could change the testicular histopathology and increased the number of spermatogenic cells of mice (*Mus musculus*) with *swim stressor* as the excessive physical treatment.

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